International Research Journal of Pharmacy and Medical Sciences
ISSN (Online): 2581-3277

Cytotoxic Activity of *Piper cubeba*: A Review

Bona Lovika[1], Fitra Fauziah[2]*, Rezlie Bellatasie[3]
[1, 2, 3]Department of Pharmacology and Clinical Pharmacy, School of Pharmaceutical Science (STIFARM) Padang, West Sumatra, Indonesia, 25147
For correspondence; Tel. +(62)85274466344, Email: fitrafauziah @ stifarm-padang.ac.id

***Abstract*—** *Several studies show P. cubeba has cytotoxic activity and has the potential to be used in cancer therapy. This article aims to review the cytotoxic activity of P. cubeba. We collected research articles from several databases, including PubMed, Sciencedirect, and Google Scholar. The keywords used are "Anticancer" OR "Cytotoxic" AND "P. cubeba". The cytotoxic activity of P. cubeba has been tested on several cancer cells lines including MCF-7, HT29, MDA-MB-468, MDA-MB-231, A549, K562, SiHa, KB, HCT116, HT29, and HTC, mainly using MTT methods. The cytotoxic activity of P. cubeba belonged to the strong and toxic categories. This activity is influenced by the part of the plant used, the type of solvent and the dose tested and also the presence of amide and lactone groups from (–)-cubebin.*

***Keywords*—** *Cubebin; Cytotoxic activity; Piper cubeba.*

I. INTRODUCTION

Cancer is the third leading cause of death with an increasing incidence every year [1]. The impact and consequences of cancer are enormous, especially on social and economic burdens [2]. In 2018, it is estimated that there are 18.1 million new cases of cancer and 9.6 million death from cancer [3].

Anticancer drugs have quite severe side effects when given in therapeutic doses [4]. The adverse effects are dangerous because anticancer drugs also attack normal cells of the body. Many studies have been conducted to find treatments that can reduce these side effects, one of them is to replace chemotherapy drugs with bioactive compounds from plants that have cytotoxic activity [5]. Medicines derived from plants are considered more tolerable and less toxic to normal cells [6].

P. cubeba pepper, known as cube pepper, tailed pepper, or Javanese pepper is a popular spice consumed throughout European, as well as in many other countries, including Arab, India, Indonesia, and Morocco. *P. cubeba* has been widely used since medieval times both as a cooking spice and as traditional medicine [7]. Traditionally, *P. cubeba* has been effective in treating kidney problems, vaginal discharge, fever, and diabetes [8]. Other studies have also shown the hepatoprotective activity of *P. cubeba* in mice [9]. Cubebin compounds from the *P. cubeba* plant have anti-inflammatory, antiparasitic, antibacterial, anti-tumor, and erectile dysfunction effects [3]. Cubebin contains amides and lactones which are a group of compounds that exhibit anticancer activity. The amide group had more potential for anticancer activity than the lactone group [10]. This literature review is written by collecting and identifying relevant articles on studies reporting the anticancer activity of *P. cubeba* plants. We aim to provide information regarding cytotoxic activity from *P. cubeba* and its usefulness as an anti-cancer agent.

II. METHODS

The database used includes Pubmed, ScienceDirect, and Google Scholar with keywords (in all search fields): "Anticancer" OR "Cytotoxic" AND "*P. cubeba*". The articles used in this review are at least published in the last 10 years, from January 2010 to November 2020. Articles are selected after identifying the title, abstract, and full text in sequence.

III. RESULT AND DISCUSSION

A. *P. cubeba* Plants

Piper cubeba Linn is an aromatic plant from the Piperaceae family. *P. cubeba* is generally known by various names depending on the region of origin. In English, it is usually called the tailed pepper, or java pepper. In Pakistan, Bangladesh, and India, to be precise in Urdu, Hindi, and Bengali, it is called kabab-chini. In Brazil, it is called "pimenta de Java" ("Java pepper") and in Indonesia it is called Cabe Jawa and Kamukus. This plant is known as cubeba in Arab countries [10]. The *P. cubeba* is a vine with rounded stems reaching 4-12 m long. *P. cubeba* has a single leaf with a tapered tip measuring 8-15 cm long and 3-5 cm wide. *P. cubeba* plants have compound flowers and round fruit. (Figure 1) [11].

P. cubeba plants are widely used in the treatment of various diseases [10]. One study reported that *P. cubeba* extract has weak antibacterial properties against *Escherichia coli* and *Staphylococcus aureus* [12]. *P. cubeba* fruit also act as natural antifungal agent in food [13]. Several studies reported other *P. cubeba* pharmacological activities are trypanocidal, anti-inflammatory, analgesic [14], anti-proliferative [15], and leishmanicidal activity [16].

P. cubeba has a bitter, sharp, and persistent taste, as well as a strong odor [10]. It contains a considerable group of essential oils, polyphenolic compounds, alkaloids, and other phytochemicals. *P. cubeba* volatility and aromatic odor justify its use as deodorants in cosmetics, pharmaceutical, and chemical industries and also as flavor in food industries [17]. *P. cubeba* fruit contains sesquiterpene hydrocarbons, particularly β-caryophyllene, δ-cadinene, α- and β-cubebenes, and small amounts of monoterpenes [18]. There are many secondary metabolites content of *P. cubeba* plants including alkaloids, glycosides, tannins, and flavonoids contained in crude ethanol extract of *P. cubeba* [19]. *P. cubeba* extracts

Bona Lovika, Fitra Fauziah, and Rezlie Bellatasie, "Cytotoxic Activity of Piper cubeba: A Review," *International Research Journal of Pharmacy and Medical Sciences (IRJPMS)*, Volume 4, Issue 3, pp. 12-18, 2021.

showed the presence of many essential oils, consisting of monoterpenes, esquiterpenes, germacene, cubebin, β-pinene. Terpinen-4-ol (42.41%), α-copaene (20.04%), and γ-elements (17.68%), with terpinen-4-ol reported to have antioxidant activities [8], [20].

Fig. 1. *P. cubeba* leaves (A), flowers (B) and fruit (C) [17]

B. Cytotoxic Activity

Based on searches from Pubmed, Sciencedirect, and Google Scholar, there are six relevant articles based on screening of article titles, abstracts, and full text related to the cytotoxic activity of *P. cubeba*. The extracts used from each of the articles reviewed included phosphate buffer saline (PBS) extract [21], methanol crude extract and dichloromethane crude extract [22], [23], and cubebin isolate from *P. cubeba* seeds [24], [25], [26]. One of the isolates from *P. cubeba*, cubebin, is widely used because cubebin has an effective anticancer activity[10]. Cytotoxic test were carried out on several cancer cell lines from humans (MCF-7, HT29, MDA-MB-468, MDA-MB-231, A549, K562, SiHa, KB, HCT116, HT29), rat (HTC), and some of the studies also use normal cell lines such as MCF-12A (human) and L929 (mice) and some of the studies also use normal cell lines (MCF-12A, L929) as control and comparison.

(3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolium bromide) (MTT) is one of the most frequently used colorimetric tests in cytotoxic tests [27]. Examination with MTT has been widely applied in testing cell stimulation and inhibition [28]. The MTT method is based on the reduction reaction of the MTT reagent (3- (4,5-dimethyltiazol-2-il) -2,5-diphenyltetrazolium bromide) which is catalyzed by the enzyme succinate dehydrogenase contained by living cells. The MTT testing stage is the transfer of some test cells as many as $1x10^4$ cells / well in complete culture media consisting of fetal bovine serum (FBS) as the main cell nutrient, penicillin-streptomycin as contaminant prevention [29].

IC_{50} is a parameter used to show the potential of a compound as toxic material. The IC_{50} value shows the concentration level that results in 50% inhibition of cell proliferation and shows the potential for toxicity of a compound to cells. The smaller the IC_{50} value, the toxic the compound is. This value is a benchmark for conducting observational tests of cell kinetics (Anggraini, 2008). IC_{50} value in ppm or µg/ml indicates the level of toxicity and can be categorized as follows: IC_{50} <30 µg/mL considered very toxic, IC_{50} 30-1000 µg/mL considered toxic, and IC_{50} > 1000 µg/mL categorized as non-toxic [30].

From ethnopharmacological studies, *P. cubeba* is a medicinal plant known to have cytotoxic activity and is quite widely prescribed by herbalists in Morocco. The PBS extract from *P. cubeba* seeds at a concentration of 500 µg/ml had cytotoxic activity against MCF-7 and HT29 with IC_{50} values of 34.2 µg/ml and 70 µg/ml, respectively, and could be categorized as toxic [21].

Two studies conducted in Thailand tested the cytotoxic activity of dichloromethane and methanol extract of *P.cubeba against* three breast cancer (MCF-7, MDA-MB-468, MDA-MB-231), normal breast cell lines (MCF-12A), and normal fibroblast (L929). The difference lies in the parts of the plant used, namely fruit [22]. and seed [23]. The results showed that the methanol extract had lower IC_{50} values in MCF-7 and MDA-MDB-486 cancer cell lines compared to dichloromethane extract which means it has better cytotoxic activity. Meanwhile, for MDA-MDB-231 cancer cells, dichloromethane extract had better cytotoxic activity compared to methanol extract [22]. The IC_{50} value of both methanol and dichloromethane extracts is classified in the toxic category (30-1000 µg/ml) and can be seen in table 1.

TABLE 1. An overview of the cytotoxic activity of *P. cubeba*

Type of extract/ active compound	Part of plant	Dose/ Concentration	Method	Cancer cell lines	Result	Ref.(s)
Phosphate buffer saline (PBS) extract	Seeds	500 µg/ml	MTT assay	MCF7 and HT29	IC_{50} • MCF-7 = 34.2 µg/ml • HT29 = 70 µg/ml.	[21]
(–)-cubebin	Seeds	0.028 µM, 0.28 µM, 2.8 µM, 28 µM, and 280 µM	MTT assay	HTC	280 µM cubebin was cytotoxic after 24, 48 and 72 h exposure, but not mutagenic at 0.28 µM, 2.8 µM and 28 µM after 26 hours.	[24]
Methanol crude extract and Dichloromethane	Fruit	0-80 µg/ml*	MTT assay	Normal breast cell (MCF-12A), and Breast cancer cell lines (MCF-	IC_{50} of Methanol crude extract • MCF-7= 26.63 ± 0.47 µg/ml, • MDA-MB-468= 22.95 ± 2.09 µg/ml,	[22]

crude extract				7, MDA-MB-468, MDA-MB-231)	• MDA-MB-231= 59.19 ± 3.97 µg/ml, • MCF-12A= > 80 µg/ml IC_{50} of Dichloromethane crude extract • MCF-7= 64.41 ± 1.61 µg/ml • MDA-MB-468= 40.82 ± 0.33 µg/ml • MDA-MB-231= 32.98 ± 1.01 µg/ml • MCF-12A= > 80 µg/ml	
Methanol crude extract and Dichloromethane crude extract	Seeds	**	MTT assay	Normal breast cell (MCF-12A), normal fibroblast (L929), and breast cancer cell lines (MCF-7, MDA-MB-468 and MDA-MB-231).	IC_{50} of Methanol crude extract • MCF-7= 22.31 ± 0.83 µg/ml • MDA-MB-468= 21.84 ± 1.60 µg/ml • MDA-MB-231= 65.12 ± 5.98 µg/ml • MCF-12A= > 80 µg/ml IC_{50} of Dichloromethane crude extract • MCF-7= 62.20 ± 0.55 µg/ml • MDA-MB-468= 54.81± 0.13 µg/ml • MDA-MB-231= 35.71± 5.73 µg/ml • MCF-12A= >80 µg/ml IC_{50} *P. cubeba* Fractions I Fraction B • MCF-7= 10.46 ± 1.28 µg/ml • MDA-MB-468= 12.90 ± 1.64 µg/ml • MDA-MB-231= 17.54 ± 1.72 µg/ml • MCF-12A= ND* Fractions C • MCF-7= 2.72 ± 0.28 µg/ml • MDA-MB-468= 3.77 ± 0.43 µg/ml, • MDA-MB-231= 4.03 ± 0.88 µg/ml • MCF-12A= 13.69 ± 2.36 µg/ml Fractions D • MCF-7= 7.09 ± 0.13 µg/ml • MDA-MB-468= 10.16 ± 1.00 µg/ml • MDA-MB-231= 20.45 ± 0.48 µg/ml • MCF-12A= ND* Fractions E • MCF-7= 4.37 ± 1.05 µg/ml • MDA-MB-468= 7.05 ± 2.76 µg/ml • MDA-MB-231= 13.48 ± 1.65 µg/ml • MCF-12A= ND Fractions F • MCF-7= 15.53 ± 0.15 µg/ml • MDA-MB-468= 26.52 ± 0.61 µg/ml • MDA-MB-231= 46.69 ± 6.84 µg/ml • MCF-12A= ND* • IC_{50} *P. cubeba* Fractions from fraction C Fractions CA • MCF-7= >80 µg/ml • MDA-MB-468= 47.21 ± 4.51 µg/ml • MDA-MB-231= >80 µg/ml • MCF-12A= ND* • L929= >80 µg/ml Fractions CB • MCF-7= 61.70 ± 6.61 µg/ml • MDA-MB-468= 23.10 ± 1.99 µg/ml • MDA-MB-231= 35.97 ± 0.54 µg/ml • MCF-12A= ND* • L929= > 80 µg/ml Fractions CC • MCF-7= > 80 µg/ml • MDA-MB-468= 58.56 ± 2.50 µg/ml • MDA-MB-231= >80 µg/ml • MCF-12A= ND* • L929= >80 µg/ml Fractions CD • MCF-7= >80 µg/ml • MDA-MB-468= 22.04 ± 2.57 µg/ml • MDA-MB-231= 45.03 ± 5.27 µg/ml • MCF-12A= ND* • L929= > 80 µg/ml	[23]

Bona Lovika, Fitra Fauziah, and Rezlie Bellatasie, "Cytotoxic Activity of Piper cubeba: A Review," *International Research Journal of Pharmacy and Medical Sciences (IRJPMS)*, Volume 4, Issue 3, pp. 12-18, 2021.

					Fractions CE • MCF-7= 2.69 ± 0.09 µg/ml • MDA-MB-468= 2.97 ± 0.15 µg/ml • MDA-MB-231= 3.98 ± 0.12 µg/ml • MCF-12A= 2.91 ± 0.15 µg/ml • L929= 4.17 ± 0.77 µg/ml Fractions CF • MCF-7= 25.95 ± 3.24 µg/ml • MDA-MB-468 =26.62 ± 4.03 µg/ml • MDA-MB-231= 32.68 ± 2.09 µg/ml • MCF-12A= ND • L929 = 55.49 ± 0.91 µg/ml Fractions CG • MCF-7= >80 µg/ml • MDA-MB-468 = >80 µg/ml • MDA-MB-231= >80 µg/ml • MCF-12A= ND • L929= > 80 µg/ml	
(–)-cubebin	Seeds	**	MTT assay	A549, K562, SiHa, KB, HCT116, and HT29	(–)-cubebin (**1**) • A549 = 8.30 ± 0.16 µM • K562 = 8.66 ± 0.43 µM • SiHa = >100 µM • KB = 8.16 ± 0.41 µM • HCT116 = 45.06 ± 3.7 µM • HT29 = 45.2 ± 0.87 µM (–)-Dihydrocubebin (**2**) • A549 = 75.55 ± 1.1 µM • K562 = 30.17 ± 5.65 µM • SiHa = >100 µM • KB = 7.82 ± 0.38 µM • HCT116 = 85.32 ± 6.4 µM • HT29 = >100 µM (–)-Cyclic ether (**3**) • A549 = 52.86 ± 6.9 µM • K562 = 7.94 ± 0.45 µM • SiHa = >100 µM • KB = 73.88 ± 1.3 µM • HCT116 = >100 µM • HT29 = >100 µM (–)-hinokinin (**4**) • A549 = 7.86 ± 0.54 µM • K562 = 9.07 ± 0.41µM • SiHa = 68.4 ± 4.0 µM • KB = 7.68 ± 0.53µM • HCT116 = 72.58 ± 6.2µM • HT29 = 35.7 ± 1.23 µM Amide derivative (**5a**) • A549 = 6.61 ± 0.42 µM • K562 = 8.37 ± 0.19 µM • SiHa = 91.50 ± 0.31 µM • KB = 9.17 ± 0.26 µM • HCT116 = 46.06 ± 1.6 µM • HT29 = 51.1 ± 0.90 µM IC_{50} value of compounds 5a-6e **5a** • A549 = 6.61 ± 0.42 µM • K562 = 8.37 ± 0.19 µM • SiHa = 91.50 ± 0.31 µM • KB = 9.17 ± 0.26 µM • HCT116 = 46.06 ± 1.6 µM • HT29 = 51.1 ± 0.90 µM **5b** • A549 = 10.85 ± 2.1 µM • K562 = 8.60 ± 1.35 µM • SiHa = >100 µM • KB = 9.16 ± 1.1 µM • HCT116 = 93.51 ± 3.1 µM • HT29 = 68 ± 1.56 µM	[25]

Bona Lovika, Fitra Fauziah, and Rezlie Bellatasie, "Cytotoxic Activity of Piper cubeba: A Review," *International Research Journal of Pharmacy and Medical Sciences (IRJPMS)*, Volume 4, Issue 3, pp. 12-18, 2021.

					5c • A549 = 8.94 ± 0.96 µM • K562 = 7.76 ± 0.24 µM • SiHa = 80.0 ± 3.34 µM • KB = 33.8 ± 0.41 µM • HCT116 = 34.10 ± 6.2 µM • HT29 = 24.6 ± 1.11 µM **5d** • A549 = 7.28 ± 0.35 µM • K562 = 7.91 ± 0.24 µM • SiHa = 57.46 ± 9.8 µM • KB = 7.09 ± 0.61 µM • HCT116 = 7.83 ± 1.2 µM • HT29 = 58.9 ± 0.87 µM **5e** • A549 = 8.75 ± 0.71 µM • K562 = 48.84 ± 4.19 µM • SiHa = 95.82 ± 7.6 µM • KB = 8.94 ± 0.42 µM • HCT116 = 66.83 ± 6.4 µM • HT29 = 23.8 ± 0.97 µM **5f** • A549 = 7.28 ± 0.39 µM • K562 = 7.98 ± 0.56 µM • SiHa = 25.32 ± 0.87 µM • KB = 80.5 ± 6.5 µM • HCT116 = >100 µM • HT29 = 25.5 ± 0.78 µM **5g** • A549 = >100 µM • K562 = 8.86 ± 0.16 µM • SiHa = 73.70 ± 8.9 µM • KB = 19.2 ± 0.51 µM • HCT116 = > 100 µM • HT29 = 64.91 ± 0.10 µM **5h** • A549 = >100 µM • K562 = >100 µM • SiHa = >100 µM • KB = 6.38 ± 0.35 µM • HCT116 = >100 µM • HT29 = 52.1 ± 1.90 µM **5i** • A549 = >100 µM • K562 = 87.38 ± 11.3 µM • SiHa = 85.18 ± 2.7µM • KB = 7.96 ± 0.45 µM • HCT116 = 45.19 ± 4.2 µM • HT29 = 51.1 ± 2.1 µM **6a** • A549 = 45.49 ± 2.9 µM • K562 = 8.69 ± 0.18 µM • SiHa = 33.35 ± 2.6 µM • KB = 9.13 ± 0.13 µM • HCT116 = >100 µM • HT29 = 72.57 ± 0.46 µM **6b** • A549 = 58.61 ± 7.5 µM • K562 = 8.88 ± 0.18 µM • SiHa = >100 µM • KB = 7.36 ± 0.38 µM • HCT116 = 65.43 ± 3.3 µM • HT29 = 71.8 ± 0.13 µM **6c** • A549 = >100 µM • K562 = >100 µM • SiHa = >100 µM • KB = 8.95 ± 1.0 µM	

Bona Lovika, Fitra Fauziah, and Rezlie Bellatasie, "Cytotoxic Activity of Piper cubeba: A Review," *International Research Journal of Pharmacy and Medical Sciences (IRJPMS)*, Volume 4, Issue 3, pp. 12-18, 2021.

					• HCT116 = >100 μM • HT29 = 72.1 ± 0.99 μM **6d** • A549 = >100 μM • K562 = 9.15 ± 0.45 μM • SiHa = 96.26 ± 3.9 μM • KB = 62.57 ± 3.4 μM • HCT116 = 64.04 ± 5.3 μM • HT29 = 66.55 ± 0.89 μM **6e** • A549 = > 100 μM • K562 = > 100 μM • SiHa = 89.29 ± 3.6 μM • KB = 7.71 ± 0.78 μM • HCT116 = 67.70 ± 0.97 μM • HT29 = 66.21 ± 0.08 μM	
(–)-cubebin	Seeds	2.8 μM, 28 μM, and 280 μM	MTT assay	Adenocarcinoma cells (HT29)	Cubebin is cytotoxic at concentrations 280 μM only, while not cytotoxic at 2.8, 14, or 28 μM	[26]

* Not spesifically mention for *P. cubeba*
** Not
ND = Not Determined

Research using the same two extract, but from the fruit parts of *P. cubeba* also showed that methanol extract had stronger cytotoxic activity compared to dichloromethane extract, especially in MCF-7 and MDA-MDB-468 cancer cell lines. The methanol extract of *P. cubeba* was further investigated and separated by column chromatography to produce six fractions (A-F). Of the six fractions, fraction C showed the strongest cytotoxic activity against all types of cancer cells. The C fraction was further separated into 7 fractions (CA-CG), and the CE fraction showed the strongest cytotoxic activity. Fraction of CE also inhibits the proliferation of MCF-7, MDA-MDB-468, and MCF-12A cells. This cytotoxic effect is selective and mediated by the induction of apoptosis. The methanol extract and its fraction which showed the most cytotoxic activity were analyzed for their structure by 'H-NMR. The results of the 'H-NMR profile indicate the presence of long hydrocarbon chains activity [23].

The dose of (–)-cubebin tested also affected its cytotoxicity effect. (–)-cubebin isolated from *P. cubeba* seeds previously macerated with 96% ethanol tested on HTC *Rattus norvegicus* and human HT29 cancer cell line. The results showed that low doses (0.28 μM, 2.8 μM and, 28 μM) did not show cytotoxic and mutagenic activities, while high dose (280 μM) showed cytotoxic activity in HTC from rat cells (Niwa *et al*., 2013). This result is also supported by other studies that low doses (–)-cubebin up to 28 μM do not cause cytotoxicity, mutagenicity, apoptotic cell death, or enhanced growth observed in human HT29 cells. At high doses, (–)-cubebin 280 μM reduces cell viability by 50% in 24 hours and categorized as cytotoxic [26].

Another thing that affects the cytotoxic activity of (–)-cubebin is the presence of amide and lactone groups. (–)-cubebin is the highest content of *P. cubeba*. Research by Rajalekshmi *et al*., in 2016 regarding (–)-cubebin and its chemical diversification activity on six cancer cells using the MTT method showed that differences in side groups also affected their cytotoxic activity. Lactol ring of (–)-cubebin (**1**) was converted into four different functionalities, (–)-dihydrocubebin (**2**), **3**, (–)-hinokinin (**4**), and **5a**. Several compounds showed strong cytotoxic activity including compounds **5a**, **4**, and **1** on the A549 cancer cell lines; compounds **3**, **5a**, **1**, and **4** on K562 cancer cell lines; compounds **4**, **2**, **1**, and **5a** on KB cancer cell lines. In SiHa, HCT116, and HT29 cancer cell lines, the IC_{50} of all compounds is in the range of the toxic group.

Initial investigations showed that compounds having a lactone ring (**4**) and a free amide group (**5a**) had better cytotoxic activity, followed by a parent compound (–)-cubebin. Then, compound **5a** was synthesized and nine new derivatives (**5a-5i**) were produced. The **5a**-**5e** derivative is then oxidized to succinimide derivatives (**6a**-**6e**). All of these compounds were then tested for their cytotoxic activity and the results showed that several derivative compounds had better activity than the parent compounds (–)-cubebin and (–)-hinokinin. Compounds **5a, 5c, 5h** were the most effective for for A549, K562, KB cancer cell lines. Meanwhile, for SiHa, HCT116 and HT29 cancer cell lines, the IC_{50} values were still in high range. These results indicate that ethyl phenyl substituted and benzyl substituted amides with para substituents are more active and provide a good cytotoxic activity. For succinimide derivatives (**6a**-**6e**), its cytotoxic activity is poor compared to the parent compound, except for K562 and KB cancer cells. From the results of morphological examinations, it was found that cell death was induced by apoptosis pathway [25].

Based on this review, it can be seen that the cytotoxic activity of *P. cubeba* is varied between parts of the plant used in the study. Secondary metabolites were formed from primary metabolites through various metabolic pathways that were influenced by several environmental factors. These factors such as light, temperature, pH, altitude, and temperature will affect the phytochemical content. The phytochemical content resulting from secondary metabolites such as flavonoids and beta carotene from a plant, of course, will also be different in each region, influenced by these environmental factors [31]. Secondary metabolite compounds in plants are usually distributed evenly throughout the plant but in different levels [32]. The solvent used for extraction also can affect levels and

Bona Lovika, Fitra Fauziah, and Rezlie Bellatasie, "Cytotoxic Activity of Piper cubeba: A Review," *International Research Journal of Pharmacy and Medical Sciences (IRJPMS)*, Volume 4, Issue 3, pp. 12-18, 2021.

types of secondary metabolites extracted and may also affect cytotoxic activity [33]. Differences side group also can affect their cytotoxicity [25]. (–)-Cubebin contained in the fruit or seeds of the *P. cubeba* plant should be consumed with caution because cytotoxic effects are observed at high concentrations [24].

IV. Conclusion

From this review, it can be concluded that *P. cubeba* contains the main active compound, cubebin, which has cytotoxic activity. *P. cubeba* also contains other secondary metabolites such as flavonoids and alkaloids which also have anti-cancer properties. Our literature study showed that differences in plant parts, solvent, doses, and amide and lacton groups in isolated cubebin influence their cytotoxic activity.

References

[1] E. Sulistiowati, D. B. Lolong, and L. Pangaribuan, "Gambaran Penyebab Kematian Karena Kanker di 15 Kabupaten/Kota, Indonesia Tahun 2011," *Bul. Penelit. Sist. Kesehat.*, vol. 19, no. 2, pp. 119–125, 2016.

[2] G. S. Stein and K. P. Luebbers, *Cancer Prevention, Early Detection, Treatment and Recovery Second Edition*. Burlington: Wiley Blackwell, 2019.

[3] R. godoy de Lima, M. T. Barros, and R. da Silva Laurentiz, "Medicinal Attributes of Lignans Extracted from Piper Cubeba: Current Developments," *ChemistryOpen*, vol. 7, no. 2, pp. 180–191, 2018, doi: 10.1002/open.201700182.

[4] G. Mishra, "Serious Adverse Effects of Anticancer Drugs- a Review," vol. 3, no. 4, pp. 1–14, 2020.

[5] F. E. Pratama and R. F. Nuwarda, "Review: Senyawa Aktif Antikanker dari Bahan Alam dan Aktivitasnya," *Farmaka*, vol. 16, no. 1, 2018.

[6] B. Prakash, *Functional and Preservative of Phytochemicals*. London: Charlotte Cockle, 2020.

[7] A. P. . Junqueira, F. . Perazzo, G. H. . Souza, and E. L. Maistro, "Clastogenicity of Piper cubeba (Piperaceae) seed extract in an in vivo mammalian cell system," *Genet. Mol. Biol.*, vol. 30, no. 3, pp. 656–663, 2007.

[8] A. Alam, S. Ahmed, S. Husain, H. Bano, and Z. Ahmed, "Kabab Chini (Piper cubeba) & Its Healing Corollary in Unani Medicine : An Overview," *Am. J. Pharm. Heal. Res.*, vol. 1, no. 6, pp. 1–202, 2013.

[9] M. Alsaid *et al.*, "Evaluation of the effectiveness of piper cubeba extract in the amelioration of CCl4-induced liver injuries and oxidative damage in the rodent model," vol. 2015, 2015, doi: 10.1155/2015/359358.

[10] M. A. Hanif, H. Nawaz, M. M. Khan, and H. J. Byrne, *Medicinal Plants of South Asia*. Amsterdam: Susan Dennis, 2020.

[11] M. A'tourrohman and M. Ulfah, "Etnobotany Study On The Utilization Of Sirih Types (Famili: Piperaceae) In Kalijambe Village, Kecamatan Bener, Purworejo District," *Biocelebes*, vol. 14, no. 3, pp. 268–278, 2020, doi: 10.22487/bioceb.v14i3.15239.

[12] Emrizal and S. Zuraida, "Uji Aktivitas Antibakteri Ekstrak Etanol Buah Kemukus (Piper cubeba L.F) Terhadap Bakteri Escherichia coli dan Staphylococcus aureus," *J. Penelit. Farm. Indones.*, vol. 6, no. 2, pp. 66–75, 2018, [Online]. Available: http://www.epa.gov/microbes/MPN_User_Manualvs3.pdf.

[13] Z. Fazly-Ann, M. Y. Nor-Hafizah, and Y. Rukayadi, "Antifungal activity of Piper cubeba L. Extract on spoilage fungi isolated from tomato, grapes and lemon," *Food Res.*, vol. 4, no. 1, pp. 191–198, 2020, doi: 10.26656/fr.2017.4(1).278.

[14] J. K. Bastos, J. C. T. Carvalho, G. H. B. De Souza, A. H. P. Pedrazzi, and S. J. Sarti, "Anti-inflammatory activity of cubebin, a lignan from the leaves of Zanthoxyllum naranjillo Griseb," *J. Ethnopharmacol.*, vol. 75, no. 2–3, pp. 279–282, 2001, doi: 10.1016/S0378-8741(01)00171-4.

[15] J. Yam, M. Kreuter, and J. Drewe, "Piper cubeba targets multiple aspects of the androgen-signalling pathway. A potential phytotherapy against prostate cancer growth?," *Planta Med.*, vol. 74, no. 1, pp. 33–38, 2008, doi: 10.1055/s-2007-993758.

[16] H. S. Bodiwala *et al.*, "Antileishmanial amides and lignans from Piper cubeba and Piper retrofractum," *J. Nat. Med.*, vol. 61, no. 4, pp. 418–421, 2007, doi: 10.1007/s11418-007-0159-2.

[17] T. Z. Abdul-Jalil and Z. A. Nasser, "Piper cubeba: Phytochemical and pharmacological review of a routinely used spices.," *Int. J. Pharm. Res.*, vol. 12, no. November, pp. 761–768, 2020, doi: 10.31838/ijpr/2020.SP1.119.

[18] B. Salehi, Z. A. Zakaria, R. Gyawali, and S. A. Ibrahim, *Piper Species : A Comprehensive Review on Their Phytochemistry , Biological Activities and Applications*. 2019.

[19] G. Nahak and R. K. Sahu, "Phytochemical Evaluation and Antioxidant activity of Piper cubeba and Piper nigrum," vol. 01, no. 08, pp. 153–157, 2011.

[20] Y. Andriana, T. D. Xuan, T. N. Quy, H. D. Tran, and Q. T. Le, "Biological Activities and Chemical Constituents of Essential Oils from Piper cubeba Bojer and Piper nigrum L.," *Molecules*, vol. 24, no. 10, pp. 1–16, 2019, doi: 10.3390/molecules24101876.

[21] A. Daoudi, A. El, H. El, D. Bagrel, and L. Aarab, "In vitro anticancer activity of some plants used in Moroccan traditional medicine," *J. Med. Plants Res.*, vol. 7, no. 17, pp. 1182–1189, 2013, doi: 10.5897/JMPR12.1167.

[22] S. Sriwiriyajan, T. Ninpesh, T. Nasomyon, and P. Graidist, "Cytotoxicity Screening of Plants of Genus Piper in Breast Cancer Cell Lines," vol. 13, no. June, pp. 921–928, 2014.

[23] P. Graidist, M. Martla, and Y. Sukpondma, "Cytotoxic Activity of Piper cubeba Extract in Breast Cancer Cell Lines," pp. 2707–2718, 2015, doi: 10.3390/nu7042707.

[24] A. M. Niwa, J. C. Marcarini, D. Sartori, E. L. Maistro, and M. S. Mantovani, "Effects of (-)-cubebin (Piper cubeba) on cytotoxicity, mutagenicity and expression of p38 MAP kinase and GSTa2 in a hepatoma cell line," *J. Food Compos. Anal.*, vol. 30, no. 1, pp. 1–5, 2013, doi: 10.1016/j.jfca.2012.12.003.

[25] D. S. Rajalekshmi *et al.*, "Anticancer activity studies of cubebin isolated from Piper cubeba and its synthetic derivatives," no. March, pp. 2–7, 2016, doi: 10.1016/j.bmcl.2016.02.041.

[26] A. M. Niwa, N. A. De Paula, D. Campos, D. Sartori, E. L. Maistro, and L. R. Ribeiro, "Evaluation of lignan (–) -cubebin extracted from Piper cubeba on human colon adenocarcinoma," *J. Toxicol. Environ. Heal. Part A*, vol. 79, no. 2, pp. 92–100, 2016, doi: 10.1080/15287394.2015.1110067.

[27] M. Larramendy L. and S. Soloneski, *Genotoxicity A Predictable Risk to Our Actual World*. London: IntechOpen, 2018.

[28] N. T. H. Nga, T. T. B. Ngoc, N. T. M. Trinh, T. L. Thuoc, and D. T. P. Thao, "Optimization and application of MTT assay in determining density of suspension cells," *Anal. Biochem.*, vol. 610, no. April, p. 113937, 2020, doi: 10.1016/j.ab.2020.113937.

[29] L. Arifianti, Sukardiman, H. Studiawan, Rakhmawati, and L. Megawati, "Uji Aktivitas Ekstrak Biji Sirsak (Annona muricata L.) Terhadap Sel Kanker Mamalia Secara In Vitro," *J. Farm. dan Ilmu Kefarmasian Indones.*, vol. 1, no. 2, pp. 63–66, 2014.

[30] B. N. Meyer, N. R. Ferrigni, J. E. Putnam, L. B. Jacobsen, D. E. Nichols, and J. L. McLaughlin, "Brine shrimp: A convenient general bioassay for active plant constituents," *Planta Med.*, vol. 45, no. 1, pp. 31–34, 1982, doi: 10.1055/s-2007-971236.

[31] F. F. Sholekah, "Kandungan Flavonoid dan Beta Karoten Buah Karika (Carica pubescens) Daerah Dieng Wonosobo," pp. 75–82, 2017.

[32] M. Ansar, A. Rahmadani, and J. Fadraersada, "Uji Aktivitas Sub Fraksi Daun Bungur (Lagerstroemia speciosa (L) pers) Sebagai Antibakteri dan Antioksidan," *Proceeding Mulawarman Pharm. Conf.*, vol. 6, no. November, pp. 179–184, 2017, doi: 10.25026/mpc.v6i1.281.

[33] M. Verdiana, I. W. R. Widarta, and I. D. G. M. Permana, "Pengaruh Jenis Pelarut pada Ekstraksi Menggunakan Gelombang Ultrasonik Terhadap Aktivitas Antioksidan Ekstrak Kulit Buah Lemon (Citrus limon (Linn.) Burm F.)," *J. Ilmu dan Teknol. Pangan*, vol. 7, no. 4, pp. 213–222, 2018, doi: 10.24843/itepa.2018.v07.i04.p08.